Phosphodiesterase - type 3 inhibitor cilostazole potentiates cAMP generation and antiproliferative effects of treprostinil in pulmonary arterial smooth muscle cells from patients with pulmonary hypertension

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INTRODUCTION

The prostacyclin class of drugs are used to treat pulmonary arterial hypertension, and while evidence suggests they improve survival, these agents eventually stop working. Thus ways are being sort to improve their clinical efficacy. We hypothesise that prostacyclin action could be enhanced by inhibition of phosphodiesterase type 3 (PDE3), a major regulator of cyclic AMP levels in the lung, whose activity appears increased in pulmonary hypertension.1

EXPERIMENTAL APPROACHES

Distal pulmonary arterial smooth muscle cells (PASMCs) isolated from the lungs of patients (n=8) suffering from idiopathic pulmonary arterial hypertension (IPAH) and rat tail artery were used for these experiments.

- **CAMP measurement**: Cyclic AMP levels were assessed in cultured human PASMCs using ELISA kit from R&D systems (Abingdon, UK). Cells were grown in DMEM:F12 containing 10% FBS to 70% confluence before being stimulated for varying times with treprostinil (TREP; 1 μM) or in combination with 1 μM cilostazole (PDE3 inhibitor), given 1 hr prior to treprostinil. Results are expressed as pmol of cyclic AMP per mg of total protein.

- **Cell proliferation assay**: Human PASMCs from IPAH patients were grown for 24 hrs in DMEM:F12 containing 10% FBS and starved for 48 hr in media alone. Cells were then stimulated with 10% FBS + treprostinil (1μM), cilostazol (1 μM or 10 μM) or in combination. Cells were counted after 4 days using ADAM-PC cell counter (Korea).

- **Myography**: To assess whether PDE3 inhibition could potentiate relaxation to treprostinil, rat tail arteries (~2mm long) were mounted onto an isometric myograph (500A JP Trading, Denmark). After a normalisation procedure vessels were contracted with 1 μM phenylephrine followed by sequential application of increasing doses of treprostinil & cilostazol (10 μM).

RESULTS

1. Time course of cyclic AMP levels in IPAH cells

![Figure 1. Cyclic AMP levels in human PASMCs in the absence and presence of treprostinil (1µM; TREP). Levels peaked within 30min and dropped back to control thereafter.](image)

2. Effect of PDE3 inhibitor on cyclic AMP

3. Effect of PDE3 inhibitor on cell proliferation

SUMMARY AND CONCLUSION

- **Cyclic AMP levels peaked within 30min of treprostinil treatment, but surprising dropped within an hour to almost the basal value. We hypothesize this is due to the higher activity of the phosphodiesterase enzymes which break down cAMP (PDE 1, 3, 4).**

- **High activity of PDE3 was assumed as we observed that the peak CAMP level was higher with the combination of treprostinil and the PDE3 inhibitor, cilostazol than with treprostinil alone and elevation was significantly sustained for up to 24 hrs compared.**

- **High activity of PDE3 might explain in part the ineffectiveness of prostacyclins (PGI2) in long-term therapy and we propose that a PDE3 inhibitor might potentiate PGI2 effects in PAH.**

- In our study we demonstrated that cilostazol could significantly potentiate cAMP levels, antiproliferative and relaxation effects of treprostinil suggesting that this combination could be clinically beneficial for treating PAH.

- **Cilostazol is already in clinical use for intermittent claudication (peripheral vascular occlusive disease) where it increases exercise tolerance and favorably modifies the plasma lipid profile, suggesting additional beneficial effects in diseases associated with atherosclerosis2 and could be possibly a new therapeutic agent for IPAH.**

REFERENCES